We claim:

1. A lentiviral vector comprising the following elements: a nucleic acid whose sequence includes (i) a functional packaging signal; (ii) a multiple cloning site (MCS); and (iii) at least one additional element selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR, wherein the lentiviral vector is a lentiviral transfer plasmid or an infectious lentiviral particle.

2. The lentiviral vector of claim 1, wherein the vector comprises at least two elements selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

3. The lentiviral vector of claim 1, wherein the vector comprises at least three elements selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

4. The lentiviral vector of claim 1, wherein the vector comprises at least four elements selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

5. The lentiviral vector of claim 1, wherein the vector comprises a second MCS, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

6. The lentiviral vector of claim 1, wherein the vector comprises a second MCS into which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-

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enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

7. The lentiviral vector of claim 1, wherein the additional element is a second MCS.

8. The lentiviral vector of claim 1, wherein the additional element is a second MCS into which a heterologous nucleic acid is inserted.

9. The lentiviral vector of claim 1, wherein the vector has unique restriction sites for at least 4 enzymes selected from the group consisting of NotI, ApaI, XhoI, XbaI, HpaI, NheI, PacI, NsiI, SphI, Sma/Xma, AccI, BamHI, and SphI.

10. The lentiviral vector of claim 1, wherein the vector has unique restriction sites for at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or at least 13 enzymes selected from the group consisting of NotI, ApaI, XhoI, XbaI, HpaI, NheI, PacI, NsiI, SphI, Sma/Xma, AccI, BamHI, and SphI.

11. The lentiviral vector of claim 1, wherein the additional element is an HIV FLAP element.

12. The lentiviral vector of claim 1, wherein the additional element is an expression-enhancing posttranscriptional regulatory element.

13. The lentiviral vector of claim 12, wherein the expression-enhancing posttranscriptional regulatory element is a WRE.

14. The lentiviral vector of claim 1, wherein the additional element is a target site for a site-specific recombinase.

15. The lentiviral vector of claim 14, wherein the site is a loxP site.

16. The lentiviral vector of claim 1, wherein the lentiviral vector is a lentiviral transfer plasmid.

17. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less than 10 kB.

18. The lentiviral transfer plasmid of claim 16, wherein the plamid has a size of less than 9 kB.

19. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less than 8 kB.

20. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less than 7 kB.

21. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of approximately 6 kB.

22. The lentiviral vector of claim 1, wherein the lentiviral vector is an infectious lentiviral particle.

23. The lentiviral vector of claim 1, further comprising: a heterologous promoter or promoter-enhancer.

24. The lentiviral vector of claim 23, wherein the heterologous promoter or promoter-enhancer is selected from the group consisting of: the CMV promoter, the CMV promoter-enhancer, and the ubiquitin C promoter.

25. The lentiviral vector of claim 24, wherein the heterologous promoter is an inducible promoter.

26. The lentiviral vector of claim 24, wherein the heterologous promoter is a cell type specific or tissue specific promoter.

27. The lentiviral vector of claim 23, wherein the heterologous promoter is an RNA polymerase promoter.

28. The lentiviral vector of claim 27, wherein the RNA polymerase promoter is an RNA polymerase III promoter.

29. The lentiviral vector of claim 28, wherein the RNA polymerase III promoter is a U6 promoter.

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30. The lentiviral vector of claim 28, wherein the RNA polymerase III promoter is an H1 promoter.

31. The lentiviral vector of claim 27, wherein the RNA polymerase promoter is an RNA polymerase II promoter.

32. The lentiviral vector of claim 23, further comprising a second heterologous promoter or promoter-enhancer.

33. The lentiviral vector of claim 1, further comprising a heterologous nucleic acid encoding a selectable marker operably linked to a promoter.

34. The lentiviral vector of claim 1, further comprising a heterologous nucleic acid encoding a reporter molecule operably linked to a promoter.

35. The lentiviral vector of claim 34, wherein the reporter molecule is selected from the group consisting of: GFP, EGFP, dsRed, dsRed2, cyan fluorescent protein, yellow fluorescent protein, blue fluorescent protein, dsRed, dsRed2, luciferase, and aequorin.

36. The lentiviral vector of claim 34, further comprising an RNA polymerase promoter.

37. The lentiviral vector of claim 36, wherein the RNA polymerase promoter is an RNA polymerase III promoter.

38. The lentiviral vector of claim 1, wherein the lentiviral vector is a transfer plasmid, further comprising a genetic element sufficient for stable maintenance of the transfer plasmid as an episome within mammalian cells.

39. A lentiviral vector comprising an RNA polymerase III promoter.

40. The lentiviral vector of claim 39, wherein the RNA polymerase III promoter is a U6 promoter.

41. The lentiviral vector of claim 39, wherein the RNA polymerase III promoter is an H1 promoter.

42. The lentiviral vector of claim 39, further comprising a heterologous nucleic acid encoding a reporter molecule.

43. A lentiviral vector having a sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

44. A collection of at least two of the lentiviral vectors of claim 43.

45. The collection of claim 44, wherein the collection includes a vector comprising a first heterologous promoter element and a vector comprising a second heterologous promoter element different from the first promoter element.

46. The collection of claim 44, wherein the collection includes a vector comprising a first heterologous reporter gene and a vector comprising a second reporter gene different from the first reporter gene.

47. A lentiviral vector having a sequence that differs by not more than 100 nucleotides from the sequence set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

48. A collection of at least two of the lentiviral vectors of claim 47.

49. The collection of claim 47, wherein the collection includes a vector comprising a first heterologous promoter element and a vector comprising a second heterologous promoter element different from the first promoter element.

50. The collection of claim 47, wherein the collection includes a vector comprising a first heterologous reporter gene and a vector comprising a second reporter gene different from the first reporter gene.

51. A lentiviral vector having a sequence that differs by not more than X nucleotides from the sequence set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9, where X represents any number between 1 and 99, inclusive.

52. A collection of at least two of the lentiviral vectors of claim 51.

53. A collection of at least two of the lentiviral vectors of claim 51.

54. The collection of claim 53, wherein the collection includes a vector comprising a first heterologous promoter element and a vector comprising a second heterologous promoter element different from the first promoter element.

55. The collection of claim 53, wherein the collection includes a vector comprising a first heterologous reporter gene and a vector comprising a second reporter gene different from the first reporter gene.

56. A three-plasmid lentiviral expression system comprising:

(a) a first plasmid whose sequence comprises a nucleic acid sequence of at least part of a lentiviral genome, wherein the plasmid (i) contains at least one defect in at least one gene encoding a lentiviral structural protein, and (ii) lacks a functional packaging signal;

(b) a second plasmid whose sequence comprises a nucleic acid sequence of a virus, wherein the plasmid (i) expresses a viral envelope protein, and (ii) lacks a functional packaging signal; and

(c) a third plasmid whose nucleic acid sequence includes (i) a functional packaging signal; (ii) a multiple cloning site (MCS); and (iii) at least one additional element selected from the group consisting of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted; an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

57. A four plasmid lentiviral expression system comprising the three plasmid lentiviral expression system of claim 56, further comprising a fourth plasmid comprising a nucleic acid segment that encodes Rev, operably linked to a promoter.

58. A cell comprising the lentiviral vector of claim 1.

59. The cell of claim 58, wherein the cell comprises a nucleic acid or nucleic acids having sequences encoding Gag, Pol, and Env proteins.

60. A cell comprising a provirus derived from the lentiviral vector of claim 1.

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61. A transgenic animal, at least some of whose cells contain the lentiviral vector of claim 1.

62. A transgenic animal, at least some of whose cells contain a provirus derived from the lentiviral vector of claim 1.

63. A method of creating a producer cell line comprising introducing the lentiviral vector of claim 1 into a host cell, wherein the lentiviral vector is a transfer plasmid; and introducing a packaging plasmid and an envelope plasmid into the host cell.

64. A method of producing lentiviral particles comprising

(i) introducing the lentiviral vector of claim 1 into a helper cell, wherein the lentiviral vector is a transfer plasmid comprising a genetic element sufficient for stable maintenance of the plasmid as an episome in mammalian cells, into a helper cell that expresses proteins required for production of infectious lentiviral particles; and

(ii) culturing the cell for a period sufficient to allow production of lentiviral particles.

65. A method of producing lentiviral particles comprising

(i) introducing the lentiviral vector of claim 1, which lentiviral vector is a lentiviral transfer plasmid comprising a genetic element sufficient for stable maintenance of the transfer plasmid as an episome in mammalian cells, into a helper cell that expresses a protein required for production of lentiviral particles, wherein expression of the protein is under control of an inducible promoter;

(ii) inducing expression of the protein required for production of lentiviral particles; and

(iii) culturing the cell for a period sufficient to allow production of lentiviral particles.

66. A method of expressing a heterologous nucleic acid in a target cell comprising

introducing a lentiviral vector of claim 1 into the target cell, wherein the lentiviral vector comprises a heterologous nucleic acid operably linked to a promoter; and

expressing the heterologous nucleic acid therein.

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67. A method for achieving controlled expression of a heterologous nucleic acid in a cell comprising steps of:

(i) providing a modified lentiviral vector comprising a heterologous nucleic acid inserted between sites for a recombinase;

(ii) introducing the modified lentiviral vector or a portion thereof including at least the sites for the recombinase and the region between the sites into the cell and;

(iii) subsequently inducing expression of the recombinase within the cell, thereby preventing expression of the heterologous nucleic acid within the cell.

68. The method of claim 67, wherein the providing step comprises inserting the heterologous nucleic acid into a lentiviral vector between sites for a recombinase, thereby producing a modified lentiviral vector.

69. The method of claim 67, wherein the cell is a mammalian cell.

70. A method for expressing a transcript in a mammal in a cell type or tissue-specific manner comprising:

(i) delivering a lentiviral vector to cells of the mammal, wherein the lentiviral vector comprises a heterologous nucleic acid operably linked to a promoter so that transcription from the promoter results in synthesis of the transcript, and wherein the heterologous nucleic acid is located between sites for a site-specific recombinase; and

(ii) inducing expression of the site-specific recombinase in a subset of the cells of the mammal, thereby preventing synthesis of the transcript within those cells.

71. The method of claim 67 or claim 70, wherein the step of inducing the site-specific recombinase comprises introducing a vector encoding the site-specific recombinase into the cell.

72. The method of claim 67 or claim 70, wherein expression of the site-specific recombinase is under control of a cell type specific or tissue specific promoter.

73. The method of claim 67 or claim 70, wherein the sites are loxP sites and the site-specific recombinase is loxP.

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74. A lentiviral vector whose presence within a cell results in transcription of one or more ribonucleic acids (RNAs) that self-hybridize or hybridize to each other to form a short hairpin RNA or short interfering RNA that inhibits expression of at least one target transcript in the cell.

75. The lentiviral vector of claim 74, wherein the vector provides a template for synthesis of an RNA that self-hybridizes to form an shRNA that is targeted to the transcript.

76. The lentiviral vector of claim 75, wherein the shRNA comprises a loop having a sequence set forth in SEQ ID NO: 10.

77. The lentiviral vector of claim 74, wherein the vector provides a template for synthesis of complementary RNAs that hybridize with each other to form an siRNA that is targeted to the transcript.

78. The lentiviral vector of claim 74, wherein the vector comprises a nucleic acid segment operably linked to a promoter, so that transcription from the promoter results in synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an shRNA or siRNA targeted to the transcript.

79. The composition of claim 74, wherein the lentiviral vector is a lentiviral transfer plasmid.

80. The composition of claim 74, wherein the lentiviral vector is an infectious lentiviral particle.

81. The lentiviral vector of claim 74, wherein:
the shRNA or siRNA comprises a base-paired region approximately 19 nucleotides long.

82. The lentiviral vector of claim 74, wherein:
the shRNA or siRNA comprises a base-paired region and at least one single-stranded overhang.

83. The lentiviral vector of claim 74 wherein:

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the siRNA or shRNA comprises a 3' overhang consisting of at least two pyrimidines.

84. The lentiviral vector of claim 83, wherein the 3' overhang is UU.

85. The lentiviral vector of claim 74, wherein:

the shRNA or siRNA comprises a region that is precisely complementary with a region of the target transcript.

86. The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level sufficient to reduce the level of the target transcript or its encoded protein by at least about 2 fold.

87. The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level sufficient to reduce the level of the target transcript or its encoded protein by at least about 5 fold.

88. The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level sufficient to reduce the level of the target transcript or its encoded protein by at least about 10 fold.

89. The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level sufficient to reduce the level of the target transcript or its encoded protein by at least about 25 fold.

90. The lentiviral vector of claim 74, wherein the lentiviral vector comprises:

(i) a functional packaging signal;

(ii) a multiple cloning site (MCS); and

(iii) at least one additional element selected from the group consisting of: a second MCS, a second MCS into which a heterologous promoter or promoter-enhancer is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.

91. A composition comprising:

the lentiviral vector of claim 74; and

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a delivery agent that enhances delivery of the vector to cells.

92. A pharmaceutical composition comprising:
the lentiviral vector of claim 74; and
a pharmaceutically acceptable carrier.

93. A three plasmid lentiviral expression system comprising (i) a lentiviral transfer plasmid comprising a heterologous nucleic acid operably linked to a promoter, so that transcription of the heterologous nucleic acid produces one or more RNAs that self-hybridize or hybridize with each other to form an shRNA or siRNA targeted to a target transcript; (ii) a packaging plasmid; and (iii) an Env-coding plasmid.

94. A four plasmid lentiviral expression system comprising the three plasmid lentiviral expression system of claim 93, further comprising a fourth plasmid comprising a nucleic acid segment that encodes Rev, operably linked to a promoter.

95. A method of inhibiting or reducing the expression of a target transcript in a cell comprising delivering the lentiviral vector of claim 74 to the cell.

96. The method of claim 95, wherein the lentiviral vector comprises:
(i) a functional packaging signal;
(ii) a multiple cloning site (MCS); and
(iii) at least one additional element selected from the group consisting of: a second MCS, a second MCS into which a heterologous promoter or promoter-enhancer is inserted, an HIV FLAP element, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR .

97. The method of claim 95, wherein the cell is a mammalian cell.

98. The method of claim 95, wherein the cell is a primary cell.

99. The method of claim 95, wherein the primary cell is a T cell.

100. The method of claim 95, wherein the cell is a non-dividing cell.

101. The method of claim 95, wherein the cell is an embryonic stem cell.

102. The method of claim 95, wherein the cell is a single-cell embryo.

103. The method of claim 95, wherein the lentiviral vector is a lentiviral transfer plasmid.

104. The method of claim 95, wherein the lentiviral vector is an infectious lentiviral particle.

105. The method of claim 95, wherein the ribonucleic acid comprises complementary regions that self-hybridize to form a short hairpin RNA targeted to the transcript.

106. A method of reversibly inhibiting or reducing expression of a target transcript in a cell comprising steps of:

(i) delivering a lentiviral vector to the cell, wherein presence of the lentiviral vector within the cell results in synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an shRNA or siRNA that inhibits expression of the target transcript, wherein the lentiviral vector comprises a nucleic acid segment located between sites for a site-specific recombinase, which nucleic acid segment provides a template for transcription of the one or more RNAs; and (ii) inducing expression of the site-specific recombinase within the cell, thereby preventing synthesis of at least one of the RNAs.

107. The method of claim 105, wherein the cell is a mammalian cell.

108. The method of claim 105, wherein the recombinase is Cre and the sites are loxP sites.

109. The method of claim 105, wherein the lentiviral vector is a lentiviral transfer plasmid.

110. The method of claim 105, wherein the lentiviral vector is a lentiviral particle.

111. The method of claim 105, wherein the lentiviral vector provides a template for synthesis of an RNA comprising complementary portions that hybridize to form an shRNA.

112. A method for reversibly inhibiting or reducing expression of a transcript in a mammal in a cell type or tissue-specific manner comprising:

(i) delivering to the mammal a lentiviral vector whose presence within a cell results in synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an shRNA or siRNA that inhibits expression of the target transcript, wherein the lentiviral vector comprises a nucleic acid segment located between sites for a site-specific recombinase, which nucleic acid segment provides a template for transcription of the RNA; and

(ii) inducing expression of the site-specific recombinase in a subset of the cells of the mammal, thereby preventing synthesis of at least one of the RNAs within the subset of cells.

113. The method of claim 112, wherein the recombinase is Cre and the sites are loxP sites.

114. The method of claim 112, wherein the lentiviral vector is a lentiviral transfer plasmid.

115. The method of claim 112, wherein the lentiviral vector is a lentiviral particle.

116. The method of claim 112, wherein the lentiviral vector provides a template for synthesis of an RNA comprising complementary portions that hybridize to form an shRNA.

117. A method of treating or preventing infection by an infectious agent, the method comprising steps of:

administering to a subject prior to, simultaneously with, or after exposure of the subject to the infectious agent, a composition comprising an effective amount of a lentiviral vector, wherein presence of the lentiviral vector in a cell results in synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an shRNA or siRNA that is targeted to a transcript produced during infection by the infectious agent, which transcript is characterized in that reduction in levels of the transcript delays, prevents, or inhibits one or more aspects of infection by or replication of the infectious agent.

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118. The method of claim 117, wherein the lentiviral vector provides a template for synthesis of an RNA that comprises complementary portions that hybridize to form an shRNA.

119. A method of treating or preventing a disease or clinical condition, the method comprising:

removing a population of cells from a subject at risk of or suffering from disease or clinical condition;

engineering or manipulating the cells to contain an effective amount of an siRNA or shRNA targeted to a transcript, which transcript is characterized in that its degradation delays, prevents, or inhibits one or more aspects of the disease or clinical condition; and

returning at least a portion of the cells to the subject.

120. The method of claim 119 wherein:

the engineering or manipulating step comprises introducing a lentiviral vector into the cells, wherein presence of the lentiviral vector in a cell results in synthesis of one or more RNAs that self-hybridize or hybridize with each other to form an shRNA or siRNA targeted to the transcript.

121. The method of claim 119, wherein:

the cells comprise stem cells.

122. The method of claim 121, wherein:

the stem cells are peripheral blood stem cells.

123. The method of claim 119, further comprising:

expanding at least a portion of the cells in culture.

124. A kit comprising (a) a lentiviral transfer plasmid comprising a nucleic acid sequence including (i) a functional packaging signal; (ii) a multiple cloning site (MCS) into which a heterologous gene may be inserted; and (iii) at least one additional element selected from the group consisting of: a second MCS, an HIV FLAP element, a heterologous promoter, a heterologous enhancer, an expression-enhancing posttranscriptional regulatory element, a target site for a site-specific recombinase,

and a self-inactivating (SIN) LTR; and one or more of the following items: (b) a packaging mix comprising one or more plasmids that collectively provide nucleic acid sequences coding for retroviral or lentiviral Gag and Pol proteins and an envelope protein; (c) cells (e.g., a cell line) that are permissive for production of lentiviral particles such as 293T cells; (d) packaging cells, e.g., a cell line that is permissive for production of lentiviral particles and provides the proteins Gag, Pol, Env, and, optionally, Rev; (e) cells suitable for use in titering lentiviral particles; a transfection-enhancing agent such as Lipofectamine; (f) a selection agent such as an antibiotic, preferably corresponding to an antibiotic resistanc gene in the lentiviral transfer plasmid; (g) instructions for use; (h) a positive control plasmid.

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